Detection of *in vitro* S-nitrosylated Compounds with Cavity Ring-down Spectroscopy

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Introduction
Vasoconstriction → NO released by epithelial cells → Vasodilation

GSNO

Cell Membrane
Trans-nitrosylation

Cell Membrane

GSNO

Cysteine

GSH

SNO-Cys
Cystic Fibrosis

Thick mucus not eliminated by cilia

Immature CFTR Chloride Channel
Functioning CFTR Chloride Channel

GSNO

Cysteine

SNO-Cys
GSNO

Cysteine

L-leucine

SNO-Cys
Cysteine

HS\_\text{NH}_2\_\text{CO}_2\text{OH}

L-leucine

\text{NH}_2\_\text{CH}_3\text{CO}_2\text{OH}
Mass Spectrometry

Chemiluminescence
Mass Spectrometry

Chemiluminescence

Sample Prep: enzymatic digestion, LC, etc.

ionization → M/Z separation → fragmentation → M/Z separation → detection

LOD ~5 ppbV

Pan et al. Sci. Rep. 5: 8725, 2005
Mass Spectrometry

Sample Prep

Sample

enzymatic digestion, LC, etc.

ionization

M/Z separation

fragmentation

M/Z separation

detection

LOD ~5 ppbV

Chemiluminescence

\[ \text{NO} + \text{O}_3 \rightarrow \text{NO}_2 + \text{O}_2 \]

Visible light emitted
Methods
$^{14}\text{NO} \quad R(13/2)_{3/2}$

$^{15}\text{NO} \quad R(15/2)_{3/2}$
Optical cavity pressure ~20 torr

100 sccm flow rate: residence time ~10 s
Results
50, 100 and 250 μL injections of 3x serial dilutions of GSNO-15. Data convolved with exponentially modified Gaussian function to suppress noise.

Raw data calibration curve.
LOD (3σ) = 8.23 pmoles
Slope = 0.0175(2) area units/pmole
function er=errcon(x,sig,t,mu)
    t=1/t;
    er=t./2.*exp(t./2.*(2.*mu+t.*sig^2-2.*x)).*erfc((mu+t.*sig^2-x)./(sqrt(2)).*sig));
end
50, 100 and 250 $\mu$L injections of 3x serial dilutions of GSNO-15. Data convolved with exponentially modified Gaussian function to suppress noise.

Raw data calibration curve. 
LOD ($3\sigma$) = 8.23 pmoles  
Slope = 0.0175(2) area units/pmole

EMG convolved data calibration curve. 
LOD ($3\sigma$) = 0.59 pmoles  
Slope = 0.0179(3) area units/pmole
Biological Sample Measurement

Human airway epithelial cells incubated with added $^{15}$N-s-nitro-cystamine (CANO)

Two cell growth filters RSNO concentration of $10 \pm 1$ nM
Exponential decay fits of $^{15}\text{NO}$ released vs incubation time $y = y_0 + Ae^{-t/\tau}$

<table>
<thead>
<tr>
<th></th>
<th>$1/\tau$ (s$^{-1}$)</th>
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<tbody>
<tr>
<td>-leucine</td>
<td></td>
</tr>
<tr>
<td>+leucine</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>$0.01 \pm 0.5$</td>
</tr>
</tbody>
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Exponential decay fits of $^{15}$NO released vs incubation time $y = y_0 + Ae^{-t/\tau}$

<table>
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<th>Condition</th>
<th>$1/\tau$ (s$^{-1}$)</th>
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<tbody>
<tr>
<td>-leucine</td>
<td>$0.1 \pm 0.06$</td>
</tr>
<tr>
<td>+leucine</td>
<td>$0.1 \pm 0.2$</td>
</tr>
<tr>
<td>control</td>
<td>$0.01 \pm 0.5$</td>
</tr>
</tbody>
</table>
Future Work
$R(13/2)_{3/2}$
Circularly polarized light gives $\Delta M = +1$ transitions.
Coefficient values ± one standard deviation

\[ a = 1.4073 \times 10^{-9} \pm 0.0208 \]

\[ b = -0.0090108 \pm 0.0005 \]

Slope = -0.0090108

Slope = -0.010225
Coefficient values ± one standard deviation

\[ a = 1.4073 \times 10^{-9} \pm 0.0208 \]
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Slope = -0.0090108

Slope = -0.010225
Coefficient values ± one standard deviation

\[ a = 1.4073 \times 10^{-9} \pm 0.0208 \]

\[ b = -0.0090108 \pm 0.0005 \]

\[ a = -9.3506 \times 10^{-8} \pm 0.0177 \]

\[ b = -0.010225 \pm 0.000499 \]

Slope = -0.0090108

Slope = -0.010225
Coefficient values ± one standard deviation

- $a = 1.4073 \times 10^{-9} \pm 0.0208$
- $b = -0.0090108 \pm 0.0005$

180 MHz p-p Laser Frequency Modulation

Slope = -0.0090108

No Laser Frequency Modulation

Slope = -0.010225
Conclusions
We have implemented an instrument that is capable of reaching the sensitivity necessary for *in vitro* metabolic studies of s-nitrosothiols by way of cell growth medium measurements.

Additional improvements are necessary to determine s-nitrosothiol concentration directly in cultured cells.

Zeeman modulation shows better stability than laser modulation indicating we will have better sensitivity with this new system.

L-leucine has undetermined effect on CANO uptake, but control trials show CANO is being broken down by human airway epithelial cells.
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\begin{figure}
\centering
\includegraphics[width=\textwidth]{graph.png}
\caption{Coefficient values ± one standard deviation}
\begin{align*}
a &= -9.3506e-008 \pm 0.0177 \\
b &= -0.010225 \pm 0.000499 \\
\text{Slope} &= -0.010225
\end{align*}
\end{figure}
We have implemented an instrument that is capable of reaching the sensitivity necessary for *in vitro* metabolic studies of s-nitrosothiols by way of cell growth medium measurements.

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Questions?